

Herbicide Effects on Embryo Implantation and Litter Size

We are concerned over specific scientific issues reported by Cavieres et al. in the November 2002 issue of *EHP* (Cavieres et al. 2002). The paper, which has already received considerable attention in the media, presents conclusions that are not supported by the experimental design or by the data; an array of significant inconsistencies and errors are also present in the paper. Accordingly, we believe that Cavieres et al. should retract their paper or the journal should withdraw it until these problems are addressed.

In the abstract of the paper, Cavieres et al. (2002) stated that

The data, although apparently influenced by season, showed an inverted or U-shaped dose-response pattern for reduced litter size, with the low end of the dose range producing the greatest decrease in the number of live pups born. The decrease in litter size was associated with a decrease in the number of implantation sites, but only at very low and low environmentally relevant doses.

The conclusions of Cavieres et al. (2002) were based on a series of seven developmental toxicity studies. Five of the experiments used pregnant mice exposed no earlier than days 5 or 6 of gestation; therefore, treatment started after implantation, so the studies were never capable of assessing effects on implantation. In only two studies were mice exposed during or before implantation, but those studies did not include the "very low doses" that Cavieres et al. claim preferentially decreased implantation sites with an inverted U dose-response curve. Thus, the basis for the authors' conclusions is in error.

Our second concern is raised by discrepancies between the paper by Cavieres et al. (2002) and Cavieres' dissertation (Cavieres 2001). The dissertation and the paper both appear to report the results of the same experiments, as evidenced, for example, by identical tables describing analytical confirmation of the doses for the seven studies. Importantly, there are unexplained discrepancies in the numbers of animals tested and in the outcomes. For example, when the two "preimplantation plus organogenesis" studies were combined in the dissertation, the control group was not significantly different from the treated groups. However, Cavieres et al.'s Table 2 contains data in which nine animals were deleted from the four groups. The authors reported statistical significance for the low-dose group after this manipulation of the data, but they did not explain why. Although the deletion of two control animals caused an increase in the mean litter size for the controls, deletions in other groups caused a decrease in mean litter size for the treated groups. The outcome of these

two manipulations accounted for the difference between treated and control animals reported by Cavieres et al. (2002), but not in Cavieres' dissertation (Cavieres 2001).

Cavieres et al. (2002) provided no reliable data to support their conclusion that the difference between litter size and implantations was due to resorptions. They did not count deaths and cannibalizations; therefore, accurate estimates of litter size are impossible.

In their Figure 1 Cavieres et al. (2002) combined data from studies that followed critically different designs and thus should not have been combined. In Cavieres' dissertation (Cavieres 2001), litter size was reported, but it was not significantly different for the low-dose "organogenesis" animals. However, combining those animals with the "preimplantation plus organogenesis" animals in Table 1 (Cavieres 2001) created a significant difference from controls.

Cavieres et al. (2002) were not consistent in their presentation of data. In Figure 1 of their paper, Cavieres et al. show that 62 control dams were used to determine litter size, but in Table 2 they show 64 control dams for the same end point. Although the authors should not have combined the various experiments, they indicated no reason for having a different number of animals in two representations of the same end point from the same collection of experiments.

Cavieres et al. (2002) also reported potential influence of seasonality on the experiments. A more plausible explanation is typical variability between experiments and that the findings are not related to treatment. The authors did not study seasonality in a systematic manner.

Finally, one of the most prominent conclusions of Cavieres et al. (2002) is a substantial overstatement of the findings of the experiments. They compared the pattern of the "dose response" with the inverted U, but the pattern in this case was inconsistent, even in the most favorable light. When the experiments are separated, as they should be, there is no reproducible dose-response pattern. It appears that the authors used novel theories to describe inconsistent data. They also used very selective citation of the literature to support the inverted U dose-response hypothesis, without citing the many unsuccessful attempts to replicate findings supportive of the hypothesis.

In conclusion, the paper by Cavieres et al. (2002) contains numerous discrepancies and inconsistencies, as well as disagreement between the results presented in their paper and in the dissertation (Cavieres 2001) upon which this paper was based. We believe that Cavieres et al. (2002) should revise and correct their paper or it should be withdrawn.

The authors declare a real, apparent, or potential conflict of interest as defined in EHP's Instructions to Authors.

James C. Lamb
Barbara H. Neal
Michael E Ginevan
BBL Sciences
Reston, Virginia
E-mail: JCL@bbl-inc.com

James S. Bus
The Dow Chemical Company
Midland, Michigan

William M. Mahlborg
Toxicology Subcommittee
Industry Task Force II on
2,4-D Research Data
Washington, DC

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Editor's note: In accordance with journal policy, Cavieres et al. were asked whether they wanted to respond to this letter, but they did not provide a response.

Reported Seasonal Dependence of Herbicide Developmental Toxicity in Mice

In their paper published in the November 2002 issue of *EHP*, Cavieres et al. (2002) exposed pregnant mice to a commercial herbicide mixture and determined pregnancy outcomes. Separate experiments were conducted for each season of the year; the spring exposure occurred between gestation days (GD) 0 and 15, whereas in the other seasons, exposure was postimplantation (GDs 5-15). The authors concluded that, although apparently influenced by season, the results showed an inverted or U-shaped dose-response pattern for reduced litter size and reduced implantation sites. These decreases were reported to occur "only at very low environmentally relevant doses of the herbicide mixture."

These findings are of interest to us, but we are concerned about several inconsistencies in the reporting and also about the merging of different groups of data. As a result, it is not possible to reconstitute the original data for independent analysis. For

example, the numbers of observations per group are different in Cavieres et al.'s Figure 1 and Table 2, and among Tables 2, 3, and 4. It is also unclear why Cavieres et al. (2002) reported a higher number of implantation sites than the number of litter size recordings (e.g., the summer high-dose group), especially when the authors stated in the text that implantations were only recorded for a subset of litters. The authors did not explain why they analyzed implantations on the basis of covariance with final litter size. The control litter-size data [Figure 1B (Cavieres et al. 2002)] has an unusual distribution, with the mode being the most frequent. The authors tentatively rationalized their findings in terms of the chemical treatment causing either preimplantation loss or fetal death. However, preimplantation exposure occurred only in the spring group, and no significant increases in resorptions were observed in any group. Thus, in the fall, winter, and summer groups, herbicide-induced preimplantation loss could not have occurred; therefore, the reported reduced implantations and reduced litter sizes in the absence of an increase in resorptions was an effect that simply could not be the result of herbicide exposure. The situation was further confused by the imperfect correlation between litter size and implantation sites. For example, in the very low-dose summer group, a significant (23%) reduction in litter size was associated with a significant (12%) reduction in implantation sites. However, in the high-dose summer group, the nonsignificant (7%) reduction in litter size was associated with a larger, but nonsignificant (15%) reduction in implantation sites.

Although the test data were tabulated according to season and a seasonal influence

on test outcome was noted, the data were merged for all cases where implantation data existed [Figure 2 (Cavieres et al. 2002)], ignoring the individual seasonal data. These seasonal data are shown in Figure 1, in the format of the Cavieres et al.'s Figure 2 (Cavieres et al. 2002). Significant reductions in litter size are distributed across all the dose groups, with the fall data following a normal monotonic decrease in litter size (Figure 1). However, Cavieres et al. excluded these monotonic fall data from their Figure 2 because of the absence of implantation data. We suggest that a primary decision should be made regarding whether or not the data are seasonally related. If they are not, the data could be merged and analyzed as such. If they are, the merging of data (as presented by Cavieres et al. in their Figure 2) is invalid, and an explanation for the seasonal influence must be sought. In the extreme, such a seasonal influence would lead to an observed normal monotonic response in the fall and an inverted U response in the summer for the same chemical. The animals used in these experiments (Cavieres et al. 2002) were purchased and maintained for 2 weeks before the experiments began to allow them to adjust to the light–dark cycle and temperature of the animal rooms. Similar conditions would have applied in the commercial animal-breeding unit; therefore, the seasonal perceptions of the mice must have derived from the journey between the supplier and the laboratory—an unlikely proposition.

We conclude that substantial uncertainty exists regarding the origin of the litter size effects reported and that the conclusion of a low-dose inverted U-shaped dose–response curve cannot be made at this stage.

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John Ashby
Ian Pate
Helen Tinwell

Syngenta Central Toxicology Laboratory
Alderley Park, Cheshire, United Kingdom
E-mail: john.ashby@syngenta.com

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Asthma and Gulf War Exposures

As a physician who treats patients with Gulf War syndrome and multiple chemical sensitivities, and on the basis of my clinical experience, I believe that these diseases are related and reflect valid pathophysiologic and biochemical processes in the body, which have yet to be clearly defined. The article by Lange et al. on respiratory illness among Gulf War veterans (Lange et al. 2002) was industrious in trying to disprove the validity of the veterans' complaints—or at least to infer that they were likely to be psychologically derived. The study has several methodologic shortcomings that need to be made explicit.

First, the authors state that

[Current] injury and symptoms of major depression, measures that have little or no biologically plausible relationship to oil-fire exposure, were included to serve as control health outcomes.

I have no problems with using "injury" as it was operationally defined in the study. However, "depression" as defined has at least two confounding and confusing correlations: First, if currently depressed subjects were chronically depressed, their perception of "level of preparedness" could be quite low, regardless of how well they were trained, because of their ongoing insecurities and low self-esteem. Second, if, in fact, Gulf War exposures made the subjects feel chronically ill, whether this was physiologic or psychologic in origin, their inability to lead normal lives could exacerbate preexisting depression or produce the reactive depression that is often seen in chronic disabling illness. These considerations make "depression" a poor independent variable. However, its use allows Lange et al. (2002) to graph similar curves

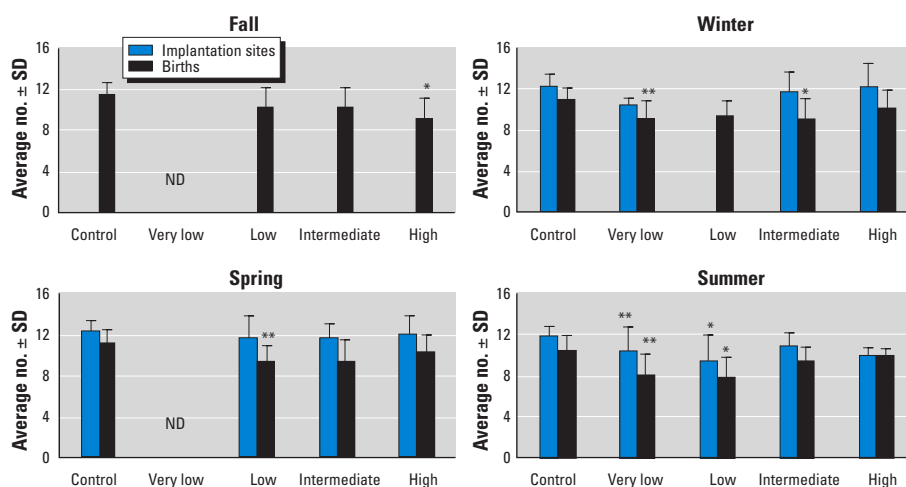


Figure 1. Graphic presentation of the data from Cavieres et al.'s Tables 2 and 4 (Cavieres et al. 2002) by season. ND, not determined. Cavieres et al. (2002) did not determine implantation numbers in the fall experiments or in the winter low-dose group or the spring very low-dose group.

* $p < 0.05$; ** $p < 0.01$; statistical assessments reported by Cavieres et al.

for asthma, bronchitis, and depression (their Figure 3), all independent of modeled exposure to oil-fire smoke. The authors ascribed all of this to "recall bias." Furthermore, a reader might easily infer that since the exposure model is "more objective" and shows no significant correlations in this study, and the veterans' complaints of asthma and bronchitis are strongly correlated with depression, all of their complaints might well be psychologic in nature.

The second methodologic shortcoming is that nowhere did Lange et al. (2002) mention that oil-fire smoke and particles are only part of the hypothesized causes of Gulf War-induced respiratory complaints. They did not mention that similar studies should be undertaken to correlate symptom reports and demographics with exposure to chemical warfare agents (or their destruction), pesticide use [such as *N,N*-diethyl-*m*-toluamide (DEET)], volatile organic hydrocarbon exposure, vaccine administration, or antichemical warfare treatment, among other hypothesized agents. Taking these factors into consideration, the impact of Lange et al.'s study is far more limited than their hypothesis and conclusions would suggest; also, their study has a built-in bias that emphasizes psychologic factors.

The author declares he has no conflict of interest.

Jeffrey B. Gordon

Ocean Beach Medical Group
San Diego, California
E-mail: paradocs2@cox.net

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Asthma and Gulf War Exposures: Response

As clearly stated in the introduction of our paper (Lange et al. 2002), there are many deployment-related exposures that have been suggested as causes for illnesses observed among veterans of the Gulf War. In our study we investigated the hypothesis that

self-reported symptoms of respiratory illnesses after the war may have been related to modeled and self-reported exposures to oil-fire smoke.

Our study (Lange et al. 2002) was not designed to address other exposures of potential significance.

In order to recognize the possibility of recall bias, our study included health conditions that had little biological plausibility for a relationship with oil-fire smoke exposure. Gordon takes issue with our use of

major depression as one of these conditions. We agree that one can construct a scenario whereby exposure to smoke in 1991 could result in recent symptoms of major depression in 1996 or injury within the past 3 months in 1996. However, these possibilities are remote compared to the pulmonary outcomes of asthma and bronchitis. Beyond recognizing the possibility of recall bias within the self-reported measures used in our study, identifying the nature of this bias or the effect of other exposures was not within the scope of our study.

The author declares he has no conflict of interest.

Peter S. Thorne

Department of Occupational and
Environmental Health
University of Iowa
Iowa City, Iowa
E-mail: peter-thorne@uiowa.edu

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Lange JL, Schwartz DA, Doebbeling BN, Heller JM, Thorne PS. 2002. Exposures to the Kuwait oil fires and their association with asthma and bronchitis among Gulf War veterans. *Environ Health Perspect* 110:1141–1146.

Communication in Emergencies

In their Commentary "Ethical Perspectives for Public and Environmental Health," Lambert et al. (2003) paraphrased a 1990 editorial that I coauthored with Lester Lave (Morgan and Lave 1990) to create a straw man against which to contrast their proposal that emergency communication should foster understanding and autonomy.

Our text (Morgan and Lave 1990) that Lambert et al. (2003) referenced reads,

There is wide, if not universal, agreement that attempts to manipulate behavior are appropriate when people are faced with large, immediate dangers. Officials are expected to issue explicit orders for action to people living in the path of a hurricane's storm surge or downwind of the spreading chlorine plume from a tank car accident, rather than just provide neutral messages for participants to weigh. Indeed, in such circumstances, if risk communications cannot get people to move out of harm's way, more intrusive measures, such as police, may be used.

Lambert et al. (2003) wrote,

In contrast, by embracing the concept of fostering autonomy, the public can formulate and share the imperative. The right to know means that people need to understand the reason behind evacuation, verbal injunctions, or barricades. Manipulation and coercion may save some lives, but they certainly do not foster understanding.

My colleagues and I are strong proponents of providing people with full understanding so that they can make independent

informed decisions. Our recent book, *Risk Communication* (Morgan et al. 2002), is entirely built on this philosophy. In it we define risk communication as

communication intended to supply laypeople with the information they need to make informed independent judgments about risks to health, safety and the environment.

Even in crisis situations, officials should try to supply complete and balanced information within the constraints of the situation. But if a chlorine plume is about to engulf my family's house, I want the state police to get them out as quickly as possible; I would be completely satisfied if the only explanation at the time was, "Madam, I'm Officer Jones of the Pennsylvania State Police. There has been a terrible chemical accident, so we must get you and your family out of here right now. We'll explain more later."

The author declares he has no conflict of interest.

M. Granger Morgan

Department of Engineering
and Public Policy
Carnegie Mellon University
Pittsburgh, Pennsylvania
E-mail: granger.morgan@andrew.cmu.edu

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Communication in Emergencies: Response

In our paper (Lambert et al. 2003), we did not advocate neutral messages in acute emergency response situations and agreed with Morgan and Lave (1990) that we have a responsibility to prevent people from entering harm's way. However, we presented an argument for fostering autonomy rather than the use of manipulation and coercion in these situations.

Hague (1929) distinguished ethical risk communication as an issue of persuasion as opposed to coercion. Faden and Beauchamp (1986) defined these extremes:

Persuasion is restricted to influence by appeal to reason, the intentional and successful attempt to induce a person, through appeals to reason, to freely accept—as his or her own—the beliefs, attitudes, values, intentions or actions advocated by the persuader.

According to Faden and Beauchamp (1986), coercion occurs if someone

intentionally and successfully influences another by presenting a credible threat of unwanted or avoidable harm so severe that the person is unable to resist acting to avoid it.

Faden and Beauchamp (1986) filled the middle ground by distinguishing forms of manipulation. They described manipulation as

the catch-all term for communication that is neither coercion nor persuasion; intentional and non-successful non-coercive influence altering the available choices of an individual, or a perception of those choices and influence that does not appeal to reason.

The essence of manipulation is having people unwittingly do what the manipulator intends for them to do. Faden and Beauchamp (1986) argued that ethical health risk communication is persuasion or, at worst, unintentional manipulation because it cannot be avoided.

Unintentional manipulation may occur through several avenues. Tversky and Kahneman (1981) demonstrated that, by framing information in particular ways (for example, in the health context, the probability of dying or living from a given procedure), the choices that people (including health care providers) make can be directed to a significant degree. Therefore, when “mere information” is presented, the presentation itself will include the danger of manipulative elements, and there is a need to recognize this dilemma and confront it. Informational manipulation occurs when the structure of perception of choices is altered by managing information to promote a desired action. Further, to varying degrees, nonsubstantive elements such as tone, manner, and order; word choice; time and setting; and the appearance, style, and charisma of the presenter can be forms of psychologic manipulation.

In his letter, Morgan has clarified his perspective and provided a communication example that does not appear to argue for manipulation. In the case of an acute emergency, Morgan would be satisfied with the following communication: “Madam, I’m Officer Jones There has been a terrible chemical accident, so we must get you and your family out of here right now. We’ll explain more later.”

This communication does allow the woman to “formulate and share the imperative,” share the officer’s urgency for evacuation, and understand the reason, (i.e., “there has been a terrible chemical accident”). Perhaps if the message also included more context, “that the plume is about to engulf your home,” a stronger argument could be made for the woman’s ability to formulate the imperative. Further, the woman may be able to spread the risk message to her friends who may not be reached by the police officers. As we argued in our paper (Lambert et al. 2003), this is a beneficial consequence of communication that fosters autonomy as opposed to manipulation. The message concludes with “we’ll explain more later.” This implies an ongoing relationship and dialogue with the woman to further foster her understanding.

In this example, however, the police officer may introduce “unintentional” manipulation. The police officer, in rushing from door to door telling people to evacuate, by his or her presence, tone, and excitement, will carry the message of the urgency of the evacuation. This will, to some degree, introduce elements of unintentional manipulation.

The communication example does not involve an attempt to manipulate the woman’s behavior or defend this approach to prevent her from being harmed. It is consistent with the argument in our paper (Lambert et al. 2003).

The author declares he has no conflict of interest.

Timothy Lambert

Department of Community Health Sciences
University of Calgary
Calgary, Alberta, Canada
E-mail: tim.lambert@calgaryhealthregion.ca

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CORRECTIONS

In “Longterm Follow-Up of Residents of the Three Mile Island Accident Area: 1979–1998” [*EHP* 111:341–348 (2003)] Talbott et al. attributed a statement to Maureen Hatch through a reference that was incorrect and would like to correct the error. In addition, there was an incorrect reference to one of her works that appeared several times.

On page 342 (second column, line 18 of the second paragraph), the Hatch et al. reference (Hatch et al. 1991) should not have been included; the corrected sentence appears below:

Additionally, individual exposures were not known but estimated. Several other researchers indicated that the exposures may have been several orders of magnitude larger than originally estimated (Wing and Richardson 2000).

Also, Talbott et al. (2003) incorrectly referenced “Hatch et al. 1991” throughout their paper; the correct reference is as follows:

Hatch MC, Beyea J, Nieves JW, Susser M. 1990. Cancer Near the Three Mile Island Nuclear Plant: Radiation Emissions. *Am J Epidemiol* 132(3):397–412.

Thus, “Hatch et al. 1991” should be replaced with “Hatch et al. 1990” throughout the text of Talbott et al.’s paper (Talbott et al. 2003); the specific locations are as follows: *a*) page 341, second column, last paragraph; *b*) page 341, third column, second paragraph; *c*) page 342, second column, second paragraph; *d*) page 343, first column, third paragraph; *e*) page 343, second column, first line; and *f*) page 347, second column, third paragraph.

The authors regret the errors.

In the March Focus article “The Earth’s Open Wounds: Abandoned and Orphaned Mines” [*EHP* 111:A154–A161 (2003)], *EHP* incorrectly characterized states’ receipt of funding under the U.S. Surface Mining Control and Reclamation Act (SMCRA) of 1977 by saying that the states of New Mexico, Colorado, Wyoming, Montana, Utah, and Washington receive SMCRA funds. In fact, although these states are eligible to receive SMCRA funds, they may or may not have applied for them. *EHP* regrets the error.